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G1B BAC B103 B201 B203
C2P PA P2E13 P2E15C P2E19B P2E19E P2E26C P2L13 P2L19C P2L19F P2L19G P2L25A P2L26C P2L28 P2L29A P3C13 P3C19F P3C28 P3C29A P7 C3H HB4

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GB 2019562 A EP 0362042 A WO 86/06493 A
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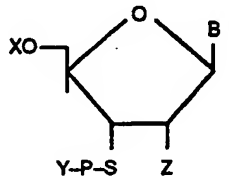
INT CL[5] C12Q, G01N

(54) DNA probe/antibody based assays and intermediates useful in the synthesis of cleavable nucleic acids for use in such assays

(57) The assay uses a labelled solid phase component (s.p.c.) which carries a member of a specific binding pair (s.b.p.) and a member of a signal producing system. A second larger and magnetic s.p.c. may also be used which carries an s.b.p. member capable of binding to the complementary s.b.p. member simultaneously with the labelled s.p.c. so as to facilitate separation of bound from unbound components. A further s.p.c. may be used in a pre-treatment to remove non-specific binding elements from the complementary s.b.p. member.

Where the target s.b.p. member is a nucleic acid, it may be captured by a thionucleotide probe immobilized on a s.p.c., and subsequently released from the s.p.c. by cleavage of the P-S bonds in the probe. Suitable oligonucleotide probes containing P-S bonds are prepared by solid phase synthesis using a thiophosphoramidite intermediate. Also disclosed are compounds of formula



where B is a DNA or RNA base and -P-Y is preferably $AO-\overset{\overset{O}{\|}}{P}-O^-$ or $R^2_2N-P-OR^1$.

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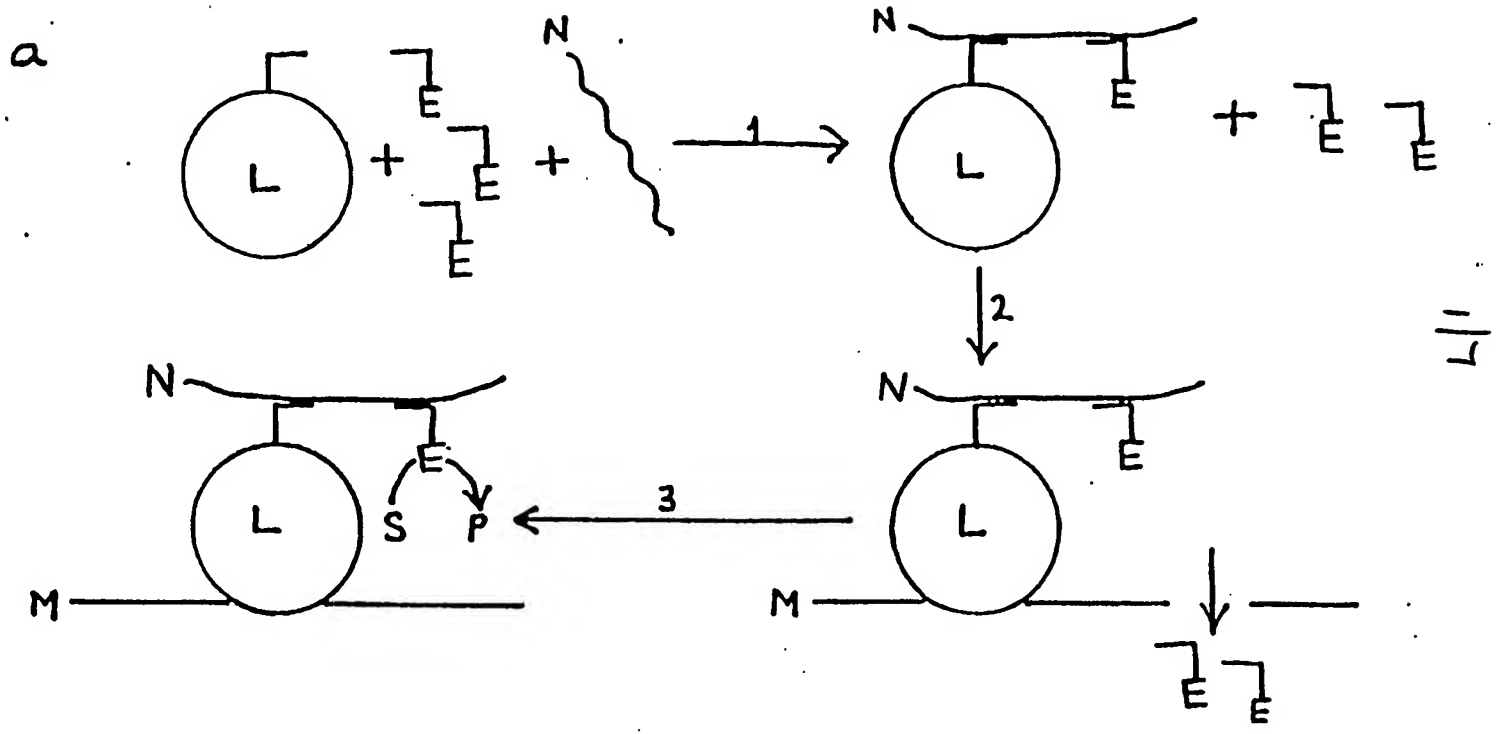


Fig. 1

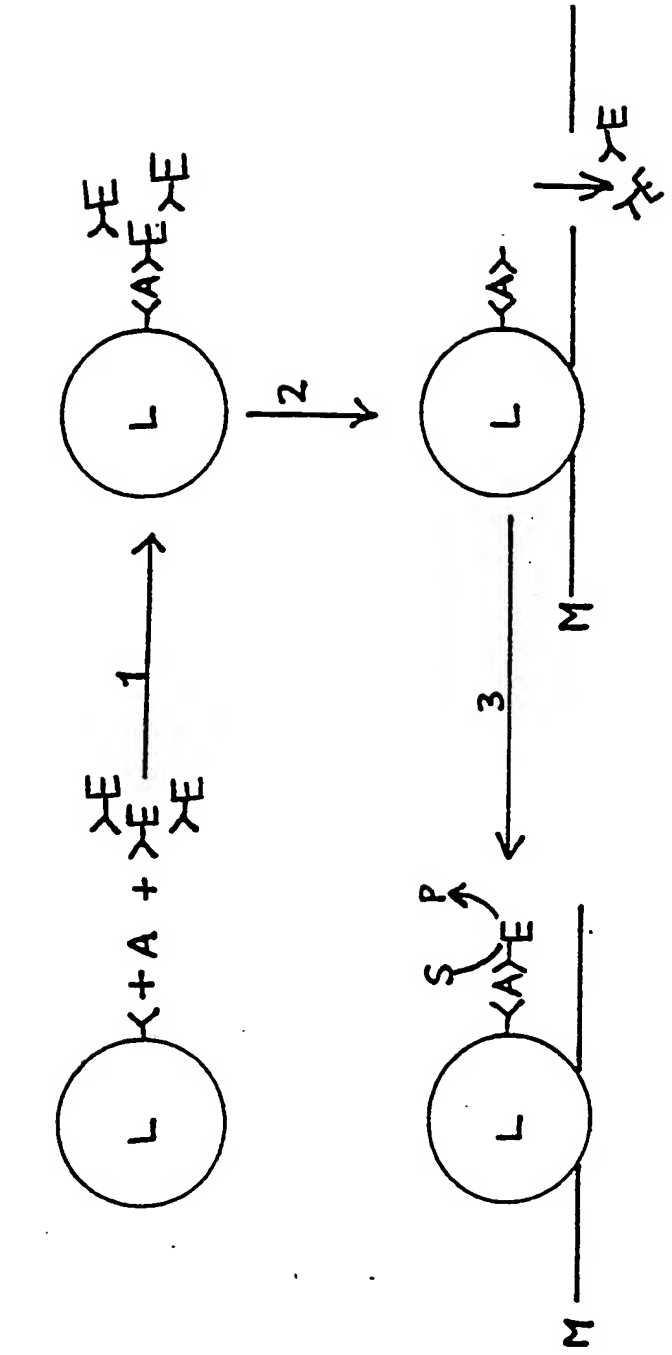


Fig. 1 cont.

b

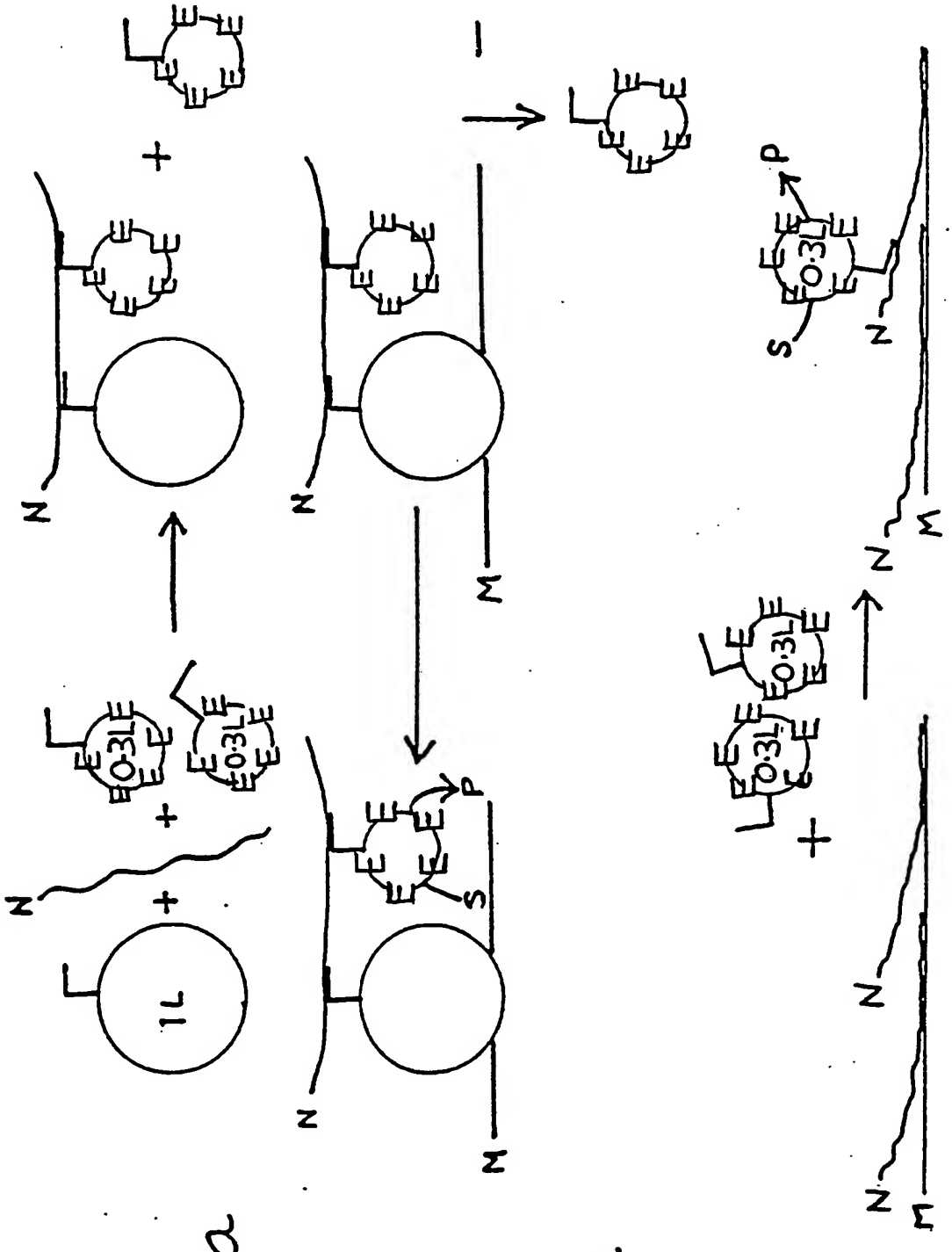


Fig. 2

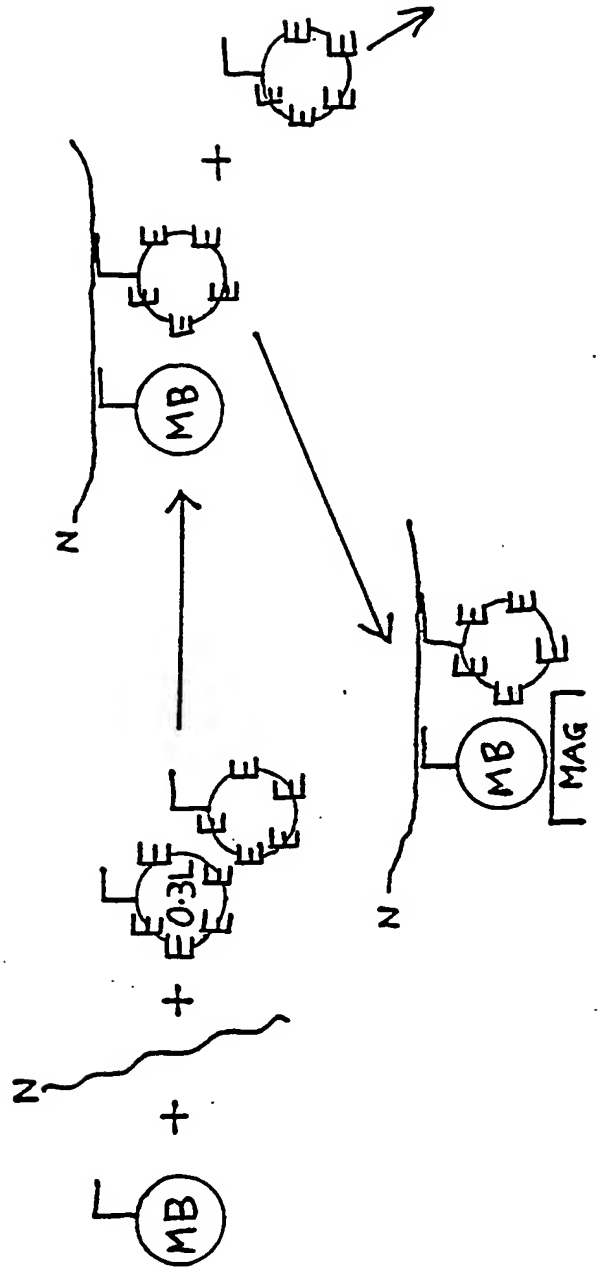


Fig. 2 contd.

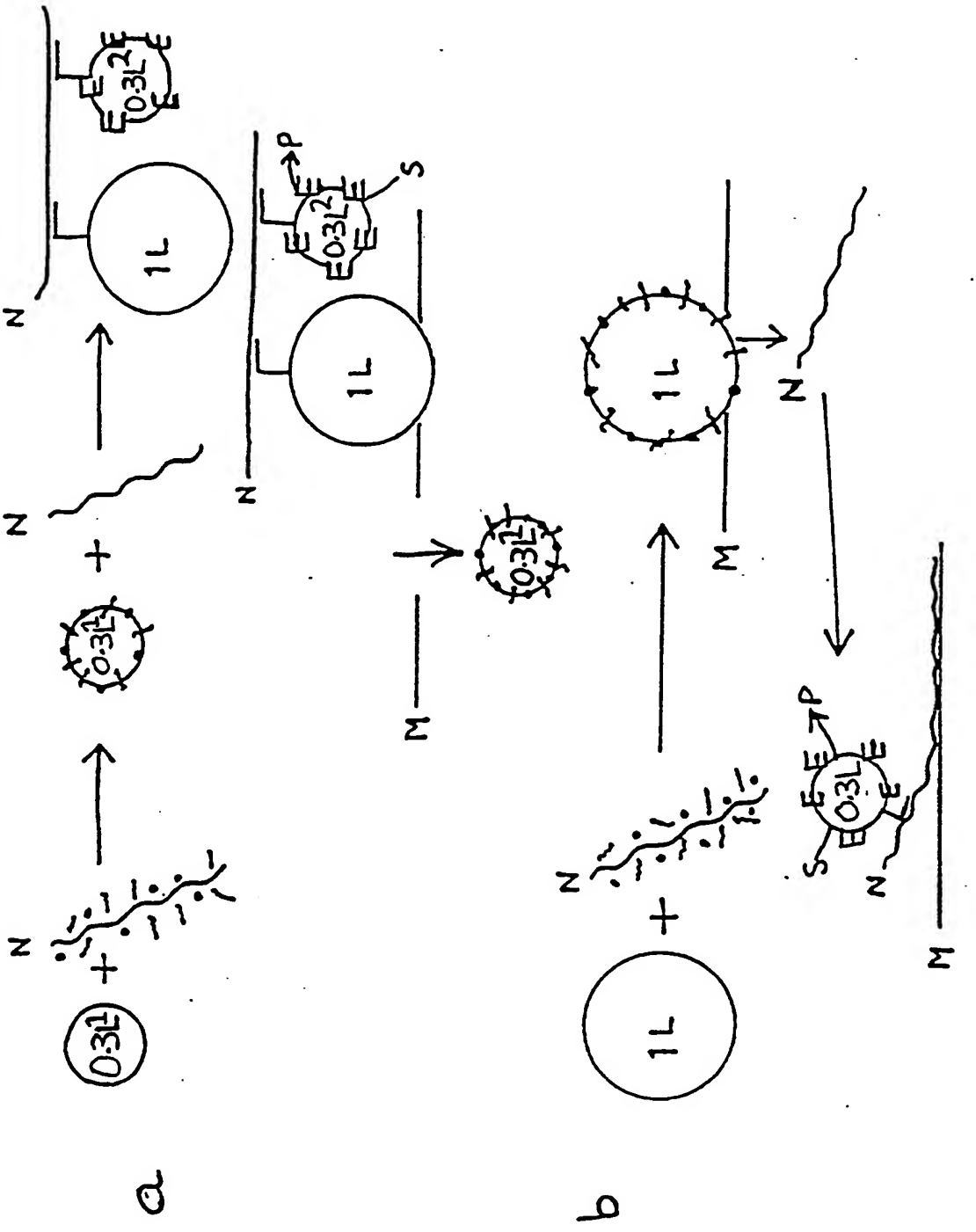


Fig. 3

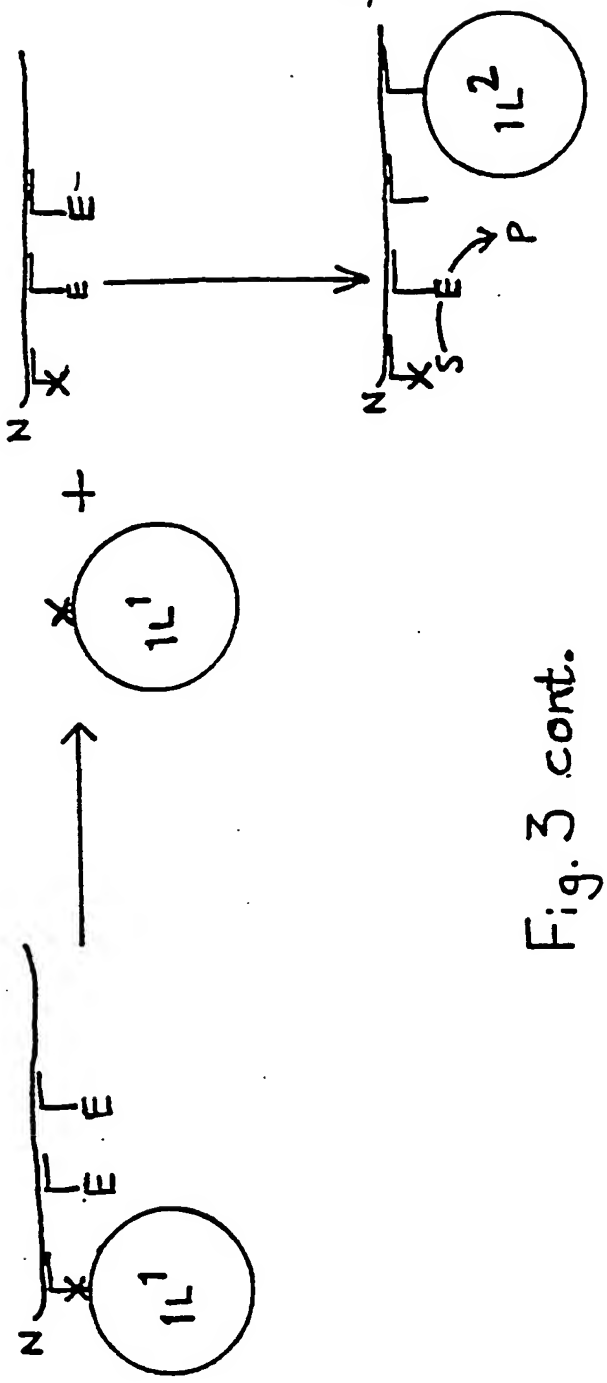


Fig. 3 cont.

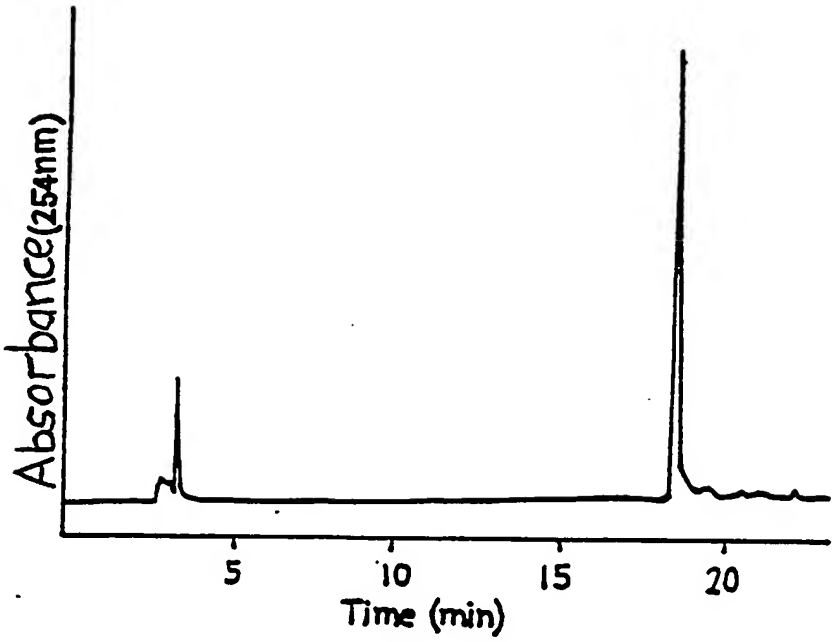


Fig. 4

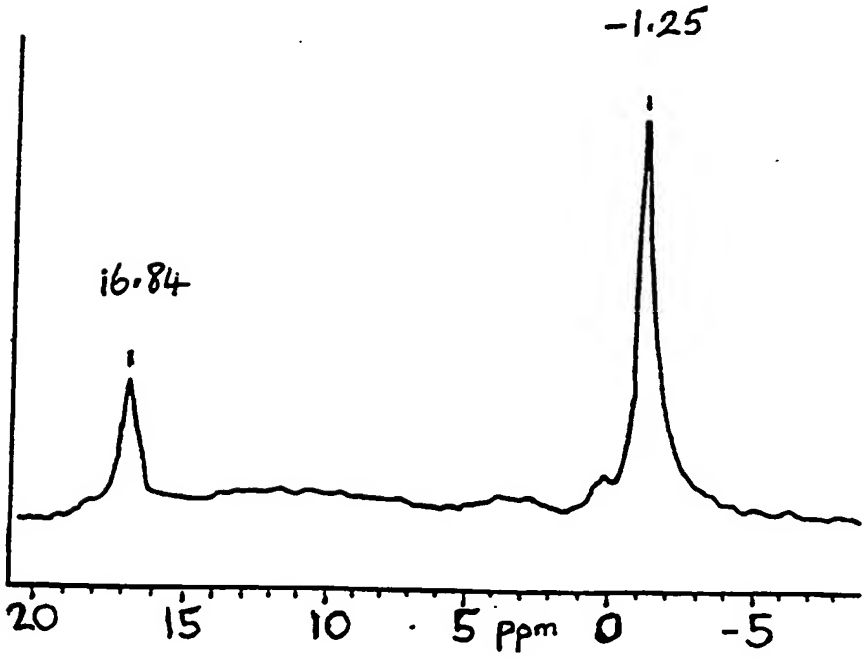


Fig. 5

DNA PROBE/ANTIBODY BASED ASSAYS AND INTERMEDIATES USEFUL IN THE SYNTHESIS OF CLEAVABLE NUCLEIC ACIDS FOR USE IN SUCH ASSAYS

This invention relates to DNA probe and antibody based assays. More particularly it relates to assays incorporating two separate (or two sets of) DNA probes or antibodies which bind simultaneously to a single target nucleic acid or antigen molecule in a "sandwich assay" format.

With existing sandwich assay technology, typically one probe/antibody is bound or captured on a solid phase ("capture probe/antibody") while the other probe/antibody is complexed with a detectable moiety such as an enzyme label or fluorescent label (labelled probe/antibody). This is illustrated in Figure 1, which shows a conventional DNA probe based sandwich assay (a) or antibody based assay (b) using a latex bead (L). In step 1, the probes/antibodies contact and bind the target nucleic acid (N)/antigen (A). In step 2, latex beads are deposited on to a membrane filter (M) which allows unbound labelled probe/antibody to pass through. In step 3, a substrate (S) is added which is converted by the detectable enzyme moiety (E) into a detectable product (P). Thus, the solid phase associated probe/antibody contacts one portion of the target nucleic acid/antigen while the other contacts a separate portion. The solid

phase is subsequently separated from the solution phase. Where target nucleic acid/antigen molecules are contacted by both capture probe/antibody and labelled probe/antibody, the labelled probe/antibody becomes indirectly attached to the solid phase. Thus, the detectable label associates with the solid phase in proportion to the amount of target nucleic acid/antigen.

The present invention provides improvements in DNA probe and antibody based assays. More particularly, the invention provides concepts and methodology incorporating the combined use of two independent solid phases for effecting DNA probe or antibody based assays which are capable of improved sensitivity and/or reduced non-specific assay signal. In addition, the invention provides novel chemical intermediates useful in the synthesis of cleavable nucleic acids to effect transfer of hybridised nucleic acids from one solid phase to another.

One limitation of the current technology is that few detectable moieties can be attached to the labelled probe/antibody. This is particularly acute with short oligonucleotide probes which present very few attachment points for detectable moieties.

One aspect of the current invention is to introduce a second solid phase to which detectable moieties and probes/antibodies are coattached such that labelled probes/antibodies are associated with multiple detectable

moieties via the second solid phase.

Assays according to this first aspect of the invention are thus capable of providing improved sensitivity.

Another limitation of current technology is that the usual solid phases employed are sources of non-specific assay signal through attachment of labelled probes/antibodies either directly or through binding to components of the sample which attach to the solid phase.

In another aspect of the current invention, one solid phase is used for removal of non-specific assay signal while the other solid phase is used for capture of sample nucleic acid/antigen.

Thus, a broad aspect of the invention resides in a DNA probe or antibody based assay employing two or more solid phases to enhance signal or reduce background.

Novel chemical intermediates for the synthesis of nucleic acids including cleavable phosphorus-sulphur bonds are included as a further aspect of the invention (see below). Chemical cleavage, as distinct for example from heat-cleavage, is particularly preferred.

The invention will be more particularly described and illustrated, with reference to the remaining figures of the accompanying drawings, wherein:

Fig. 2 shows examples of DNA probe-based assays according to one aspect of the invention.

(a) DNA probe based assay for sample nucleic acid

(N) using two sizes of latex bead (L), for example a 1µm latex bead (1L) which can be retained by, for example, a 0.8µm membrane filter (M), and a 0.3µm latex bead (0.3L) to which DNA probes and enzymes (E) are coattached and which is not retained by a 0.8µm membrane filter. The enzyme label generates a signal-producing product (P) from substrate (S).

(b) DNA probe based assay using a membrane filter (M), for immobilisation of sample nucleic acid (N), and a 0.3µm latex bead (0.3L) for specific hybridisation and introduction of enzyme (E).

(c) DNA probe based assay as in (a) using a magnetic (MB) bead, a 0.3µm latex bead (0.3L) labelled with enzyme (E) and a magnet (Mag) for attracting the magnetic beads.

Fig. 3 shows examples of DNA probe-based assays according to a second aspect of the invention.

(a) Pretreatment of sample with 0.3µm latex bead ($0.3L^1$) to attract non-specifically binding components, followed by sandwich hybridisation of sample nucleic acids (N) with 1µm latex capture bead (1L) and 0.3µm latex bead ($0.3L^2$) with coattached DNA probe and enzyme (E). The pretreated 0.3µm beads ($0.3L^1$) and unhybridised labelled beads are subsequently removed by filtration through membrane (M).

(b) Pretreatment of sample with 1µm latex bead (1L) following by filtration of the sample through a 0.45µm

filter membrane (M^1) and immobilisation of sample nucleic acids (N) onto a capture membrane (M^2). A 0.3μm bead (0.3L) with coattached DNA probe and enzyme is subsequently introduced for hybridisation to target nucleic acids.

(c) Hybridisation of target nucleic acids to a first 1μm latex bead ($1L^1$) with a cleavable DNA probe. Subsequent cleavage (X) allows release of the hybrid for capture by a second 1μm capture bead ($1L^2$) and hybridisation with a 0.3μm latex bead (0.3L) with coattached DNA probe and enzyme.

Fig. 4 shows HPLC analysis of crude DMT-d(TpTpTspTpT) (elution using a gradient (30 min) of 12 to 65% MeCN in 100 mM triethylammonium acetate pH 6.5).

Fig. 5 shows ^{31}P nmr spectrum of d(TpTpTspTpT) (5 mM oligodeoxynucleotide in D_2O).

Referring firstly to Fig. 2, in one embodiment (Figure 2a), two sizes of latex beads are employed, for example a 1.0μm capture bead and a 0.3μm labelled bead, the latter comprising coattached probe and detectable enzyme moiety. Following contact of capture and labelled probes with the target nucleic acid, the mixture is passed through a 0.8μm filter on which the 1μm latex beads are trapped while the 0.3μm beads pass through. 0.3μm beads associated indirectly with the 1μm capture beads through cohybridisation to the target nucleic acid are thus trapped on the filter membrane and, through the

detectable enzyme, give rise to a signal in proportion to the target nucleic acid. Through the contribution of many enzyme molecules per bead (optimally over 1000), target nucleic acid is detected with a greater sensitivity than previously available with enzyme attached directly to the probe.

In another embodiment of the invention (Figure 2b), target nucleic acid is immobilised onto the first (capture) solid phase and subsequently contacted with a latex bead comprising coattached probe and detectable enzyme moiety. Following a period of contact, excess beads are washed from the filter and the remaining beads detected through the detectable enzyme moiety giving rise to a signal in proportion to immobilised target nucleic acid.

In a third embodiment of the invention (Figure 2c), a magnetic capture bead is employed together with a latex bead comprising coattached probe and detectable enzyme moiety. Following contact of capture and labelled probes with the target nucleic acid, the mixture is subjected to a strong magnetic field which allows retention of the magnetic beads and any latex beads associated through cohybridisation of sample nucleic acids to magnetic and latex beads. Excess unassociated latex beads are decanted/washed away from the magnetically bound beads which subsequently give rise to a signal in proportion to the target nucleic acids.

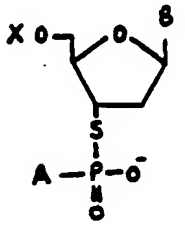
Referring next to Figure 3, in one embodiment (Figure 3a), two sizes of latex bead are used, and the sample is first contacted with, for example, 0.3μm beads which adsorb non-specifically binding components of the sample. In a subsequent step, 1μm capture beads are introduced to which sample nucleic acids bind specifically in conjunction with labelled probe. Finally, the mixture is passed through a 0.8μm filter on which 1μm capture beads are retained while 0.3μm beads pass through, thus precluding non-specific assay signal from the membrane filter.

In a second embodiment (Figure 3b), a membrane is used as the capture solid phase (cf Figure 2b), the sample is first mixed with 1μm latex beads and then passed through a 0.8μm filter thus eliminating non-specifically binding components from subsequent contact with the capture membrane and latex beads comprising coattached probe and enzyme.

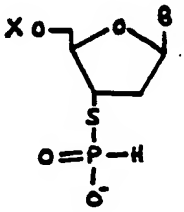
In a third embodiment (Figure 3c), the sample is first contacted with a capture latex bead to which target nucleic acids hybridise in association with nucleic acid probes. Unbound sample components and probes are then washed from the solid phase by filtration or centrifugation. In a subsequent step, the hybrid is released from the capture bead by specific cleavage of the DNA probe associated with the capture bead. This can be effected by synthesis of nucleic acid probes including

phosphorus-sulphur bonds which can be cleaved by mild oxidative hydrolysis.

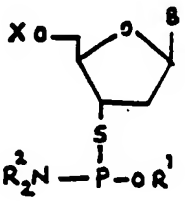
The following formulae show novel nucleotide intermediates useful for the synthesis of oligonucleotides containing cleavable phosphorus-sulphur bonds.



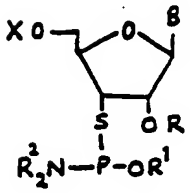
(a)



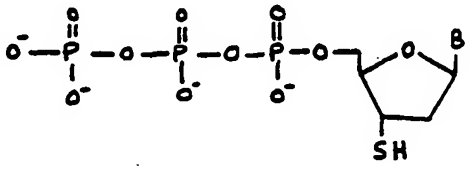
(b)



(c)



(d)



(e)

wherein:

B is one of the common heterocyclic bases of DNA or RNA or an analogue thereof.

X is a 5'-OH protecting group, preferably dimethoxytrityl

A is an aryl protecting group

R^1 is an aliphatic protecting group, preferably

cyanoethyl or methyl

R^2 is an alkyl group, preferably methyl, ethyl or propyl.

Structures (a) to (c) are particularly useful in the automated solid phase synthesis of oligodeoxynucleotides containing phosphorus-sulphur bonds, whilst (d) represents a corresponding ribonucleotide intermediate which is useful for incorporation into oligoribonucleotides. (e) represents an intermediate which is useful for enzymatic incorporation into oligodeoxyribonucleotides, particularly through reactions mediated by DNA polymerases at high temperatures (e.g. 60°C).

It will be apparent to those skilled in the art that combinations of solid phases other than those particularly described herein can be used to achieve either enhanced signal or reduced non-specific assay signal with either DNA probe based or antibody based assays.

The invention is more particularly illustrated by the following examples.

EXAMPLE 1

(a) Coattachment of Probe and Enzyme (Alkaline Phosphatase) to Beads

The 10mg of carboxylated 0.3 µm latex beads (Estapor, supplied by Rhone-Poulenc, UK) were added 1.5nmoles of 5' phosphorylated oligonucleotide of sequence 5' CCG TTC CTA CTA GAT CAG ACG ACT CCT AGT GCC GTC AGC AAA GGC TTC TA 3', 200µg of alkaline phosphatase (calf intestinal, molecular biology grade, Boehringer Mannheim) and 5mg of EDC (ethyl-dimethylaminopropyl carbodiimide) in 200µl of 40mM MOPS (morpholine propan sulphonic acid) buffer at pH6. The mixture was incubated overnight at 37°C. The latex beads were then washed through a series of centrifugations and resuspensions three times in 30% DMSO (dimethylsulphoxide) and three times in 4x SSC (four times concentrated SSC where SSC is 0.15M NaCl/0.015M sodium citrate). Washed particles were finally resuspended in 60µl H_2O and stored at 40°C.

(b) Hybridisation Detection of E. coli Ribosomal RNA

To 10mg of carboxylated 0.8µm latex beads (Estapor) were added 1.5nmoles of 5' phosphorylated oligonucleotides of sequence 5' ATT CTC ATC TCT GAA AAC TTC CGTG 3' and 5mg of EDC in 100µl 40mM MOPS, pH6. The mixture was incubated overnight at 37°C and the particles washed as in (a) above.

For hybridisation experiments, RNA was extracted from the E. coli strain DH1 (ATCC 33849) as follows. A fresh overnight suspension was inoculated into 50ml L-Broth until exponential growth to OD_{550} = 0.5 was achieved. Cells were pelleted at 10000g/4°C for 5 minutes and resuspended in 3ml of 0.2M Tris pH9, 50mM NaCl 10mM EDTA and 0.5% SDS. The solution was immediately extracted twice with 3ml of phenol (pH7 in 10mM Tris, 1mM EDTA) and once with 2ml chloroform/isoamyl-alcohol 1:1. Finally, RNA was precipitated by addition of 0.3ml 2M sodium acetate pH5.2 and 2 volumes of ethanol (held at-70°C for 30 minutes prior to centrifugation). Pellets were washed once in ice cold 70% ethanol, recentrifuged and resuspended in 3ml 50mM Tris, pH7.2, 6mM $MgSO_4$ and 0.1mM dithiothreitol, pH7.2. 50 units of DNase I (Worthington) was added and the solution was incubated at 37°C for 30 minutes. Solutions were then phenol and chloroform extracted as above and RNA ethanol precipitated. The final pellet was resuspended in 200µl water.

40µg of RNA (principally ribosomal RNA) was denatured in 50µl of water heated to 70°C for 10 minutes and placed on ice. 2.5mg of oligonucleotide conjugated 0.8µm particles and 2.5mg of oligonucleotide/alkaline phosphatase conjugated 0.3µm particles were mixed with the denatured RNA in 100µl of 4 x SSC/10% formamide and the mixture was incubated at 37°C for 2 hours. Beads

were washed 3 times in 4 x SSC and once in 1 x SSC. 2.5ml of a buffer comprising 0.5 mg bromo-chloro indoyl phosphate and 0.75mg nitro-blue tetrazolium in 0.1M Tris, pH9.5, 0.1M NaCl and 5mM $MgCl_2$ and colour allowed to develop at room temperature.

The result was the development of a strong blue colour in tubes containing RNA but not with RNA omitted.

EXAMPLE 2

Solid phase synthesis of oligonucleotides containing 3'-thiothymidine

This example details methodology for the solid phase synthesis of oligonucleotides including phosphorus-sulphur bonds through incorporation of a novel thiophosphoramidite intermediate.

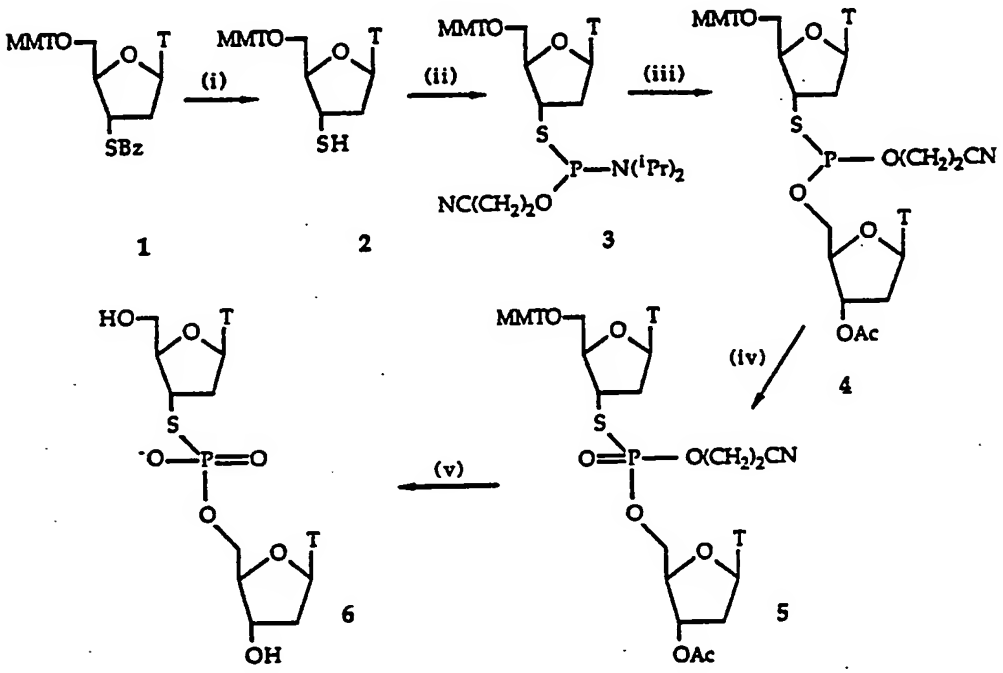
Polynucleotide analogues containing modified internucleotide linkages are receiving considerable attention as potential therapeutic agents (G.Zon, Pharmaceutical Res. 5, 539 (1988)) and tools for the manipulation of DNA (F.Eckstein and G.Gish, TIBS (Trends in Biochemical Science), 14, 97 (1989)). The phosphorothioate (F.Eckstein et al supra, and F.Eckstein, Ann. Rev. Biochem. 54, 367, (1985)) and phosphorodithioate (W.K.-D.Brill et al, J. Am. Chem. Soc., 111, 2321 (1989)) modifications are particularly attractive since they are isopolar and isosteric with the natural congener. Recently we reported the synthesis and

characterisation of 3'-thiothymidylyl(3'-5')thymidine (R.Cosstick et al, J. Chem. Soc., Chem. Comm., 992 (1988); a dithymidine phosphate analogue in which a sulphur atom replaces the 3'-oxygen atom in the phosphodiester linkage. It was noted that this compound was resistant to hydrolysis by nuclease P1, but the phosphorus-sulphur bond could be cleaved under mild oxidative conditions. This present Example describes the preparation of a 5'-O-monomethoxytritylthymidine-3'-S-thiophosphoramidite (an embodiment of compound (c) above), and its application to the solid phase synthesis of oligodeoxynucleotides containing 3'-thiothymidine.

The cleavage methodology may for example employ silver nitrate, or alternatively oxidation with iodine (R.Cosstick and J.S.Vyle, J. Chem. Soc., Chemical Communications (1988) p992-3). The intermediate thiophosphites are most efficiently oxidised using tetrabutylammonium periodate.

5'-O-monomethoxytrityl-3'-S-benzoyl-3'-thiothymidine (1) was prepared (Cosstick et al, supra). Treatment of (1) (2.4mmol) in argon saturated ethanol (240 ml) at 5°C with 10N sodium hydroxide (7 ml) gave complete debenzoylation after 50 min (see the scheme below). Silica gel column chromatography afforded 5'-O-monomethoxytrityl-3'-thiothymidine (2) in excellent yield (95%) and only minor quantities (3%) of the corresponding disulphide. Reaction of (2) with 2-cyanoethyl-N,N-

diisopropylaminophosphomonochloridite (N.D.Sinha et al, Nucleic Acids Res., 12, 4539 (1984), or preferably J.Nielson et al, Ibid., 15, 3626 (1987)) was performed under standard conditions (L.J.McBride et al, Tetrahedron Lett., 24, 245 (1983)) to give the thiophosphoramidite (3) (FAB^+ mass spectrum 731 (M+H); ^{31}P nmr ($CDCl_3$) δ 164.4 and 159.9) in a yield of 87%.

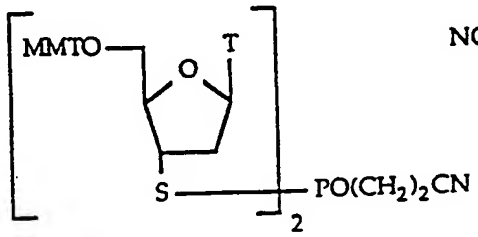


Scheme; (i) sodium hydroxide; (ii) 2-cyanoethyl-N,N-diisopropylaminophosphomonochloridite; (iii) 3'-O-acetylthymidine, 5-(p-nitrophenyl)tetrazole; (iv) oxidant; (v) t-butylamine, 80% aqueous acetic acid, conc. aqueous ammonia.

It became apparent from initial experiments that the thiophosphoramidite was less reactive under standard coupling conditions than the corresponding 3'-O-phosphoramidite. For example, under conditions previously established as giving quantitative conversion of the 3'-O-phosphoramidite to the 3',5'-dinucleoside phosphite (tetrazole, 4 equivalents; N,N-dimethylaminopyridine (DMAP) (R.T.Pon, Tetrahedron Lett., 28, 3643 (1987)), 0.8 equivalent; and 3'-O-acetylthymidine, 1.1 equivalents, in acetonitrile at room temperature for 8 min) greater than 95% (estimated by ^{31}P nmr) of (3) remained. Variations on this procedure gave different yields of the two symmetrical phosphites (7) and (8), but no resonances attributable to the two diastereomers of the desired thiophosphite (4) were observed.

The use of a more acidic activating agent such as 5-(p-nitrophenyl)tetrazole (B.C.Froehler et al, Ibid., 24, 3171 (1983)) gave, in addition to the symmetrical phosphites, ^{31}P signals corresponding to the thiophosphite (4) and conditions were developed to maximise this product. Thus, a solution of 3'-O-acetylthymidine (0.12 mmol) in acetonitrile (1.6 ml) was added dropwise over 20 min to a stirred solution of (3) (0.34 mmol) in the same solvent (0.9 ml) saturated with 5-(p-nitrophenyl)tetrazole. The symmetrical phosphites appear to result from side reactions that occur on

activation of the thiophosphoramidite. For example, treatment of (3) with 5-(p-nitrophenyl)tetrazole in the absence of 3'-O-acetylthymidine gave a significant yield of (2). This result is consistent with displacement of the 3'-thionucleoside from the phosphorus centre by the activating agent and would account for formation of both (7) and (8). Formation of (8) would also result from the analogous reaction of the thiophosphite (4).



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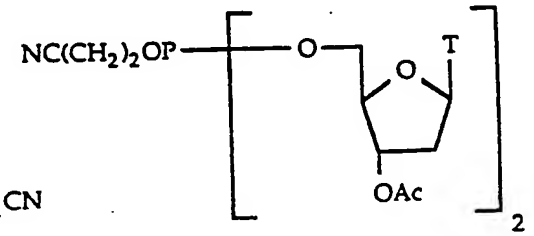

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In situ oxidation of (4) was performed by initially quenching the reaction with 2,6-lutidine (0.1 ml) followed by the addition of the oxidant either tetrabutylammonium (TBA) oxone (B.M.Tros et al, J. Org. Chem., 53, 532 (1988)), 2 equivalents, or TBA periodate (J.-L.Fourrey et al, Tetrahedron Lett., 26, 1217 (1985)), 2 equivalents, in dichloromethane. The fully protected dimer (5) ((FAB^1+ mass spectrum 930 (M+H) 952 (M+Na); 3^1P nmr ($CDCl_3$) δ 26.0 and 26.4)) was isolated in yields of 56% (under less than optimum conditions) and 75% respectively based on 3'-O-acetylthymidine. Deprotection of (5) was performed under standard conditions (see the

legend to the Scheme above).

Solid phase synthesis of d(TpTpTspTpT) (the central thymidine residue is replaced by 3'-thiothymidine) was performed on controlled pore glass (T.Atkinson and M.Smith in "Oligonucleotide Synthesis: a Practical Approach", M.J. Gait Ed., pp.35-81, IRL Press, Oxford (1984)) using a continual flow bench synthesizer (Omnifit manual bench synthesizer see B.S. Sproat and M.J. Gait, Ibid., pp. 83-115) according to the protocol outlined in the Table.

Table. Reaction cycle

Reaction	Reagents	Time (min)
Capping	6.5% DMAP in THF/acetic anhydride/2,6-lutidine (75:15:10)	3
Wash	Acetonitrile (MeCN)	2
Wash	1,2-Dichloroethane (DCE)	1
Deprotection	5% Trichloracetic acid/DCE	3
Wash	DCE	1
Wash	MeCN	4
Couple	Stop flow	
Wash	MeCN	1
Oxidation	1.0 mM TBA periodate in DCM/ MeCN/2,6-lutidine (5:5:2)	3
Wash	MeCN	4

In a typical procedure for the introduction of the 3'-thiothymidine residue, (3) (70 µmol) was dissolved in acetonitrile (0.5 ml) saturated with 5-(p-nitrophenyl)tetrazole, and injected over a period of 8 minutes into the column containing the solid support (82 mg, capacity: 36 µmol/g). The coupling was then repeated on half this scale over a period of 4 min. The coupling efficiency was approximately 80%, as determined by monitoring the release of the trityl cations. Thymidine residues were introduced as the 5'-DMT protected 3'-O-(2-cyanoethyl)-N,N-diisopropylaminophosphites using a standard activation procedure (Atkinson et al, supra): coupling solutions containing nucleoside phosphoramidite (40 µmol) and tetrazole (125 µmol) in acetonitrile (0.45 ml) were injected into the column over a period of 6 min. Phosphate deprotection and cleavage from the support was effected by treatment with concentrated ammonia solution overnight at 47°C. The crude DMT-protected pentamer was purified by reverse phase HPLC (Figure 4) and finally, the dimethoxytrityl group was removed by a treatment with 80% aqueous acetic acid for 1 hour at room temperature. The ^{31}P nmr spectrum of pure d(TpTpTspTpT) showed resonances at 16.84 and -1.25 ppm attributable to the 3'-S-phosphorothioate and phosphate groups respectively (Figure 5). Digestion with nuclease P1 in the presence of alkaline phosphatase gave thymidine and 3'-thiothymidine in the expected ratio of 4:1, (during the

long incubation that is necessary to effect complete hydrolysis to the constituent nucleosides the 3'-thiothymidine is partially oxidised to the corresponding disulphide). It is interesting to note that under the same digestion conditions (6) is almost completely resistant to hydrolysis. Treatment of d(TpTpTspTpT) with aqueous silver nitrate (30mM) at room temperature for 1 hour, gave clean and quantitative cleavage of the phosphorus-sulphur bond to yield d(pTpT) and the silver salt of the corresponding thiol.

There was also prepared a self-complementary dodecamer d(GACGATsATCGTC) containing the recognition sequence (underlined) for the restriction endonuclease EcoRV, in which a 3'-thiothymidine residue is placed at the cleavage site. Using the restriction enzyme under conditions which gave complete hydrolysis of the natural dodecamer within 5 minutes, there was no observable cleavage of the modified dodecamer in 24 hours.

These results demonstrate that oligodeoxynucleotides containing a 3'-thiothymidine residue can be prepared from 5'-O-monomethoxytritylthymidine-3'-S-thiophosphoramidites using chemistry that is compatible with automated solid phase synthesis. The ease with which the modified linkage is cleaved in the presence of silver ions suggests that these analogues may be of considerable interest in the nicking and manipulation of DNA.

CLAIMS

1. An assay procedure in which a labelled solid phase component (spc) carries a member of a specific binding pair (sbp) and a member of a signal producing system (sps), whereby the binding of said sbp member to its complementary member and the removal of unbound labelled spc allows the detection of bound sbp members by means of the associated bound sps members.

2. An assay procedure according to claim 1 wherein a second spc carries a sbp member capable of binding, simultaneously with the sbp member capable of binding, simultaneously with the sbp member carried by the labelled spc, to the same complementary sbp member, whereby the associated second spc facilitates separating the bound labelled spc from unbound labelled spc.

3. An assay procedure according to claim 2 wherein the second spc is larger than the labelled spc.

4. An assay procedure according to claim 2 wherein the second spc is magnetic.

5. An assay procedure according to claim 2 wherein after removal of unbound labelled spc the second spc is

cleaved from the complementary sbp member, which is then captured by a further spc prior to detection by the sps.

6. An assay procedure according to any one of the preceding claims wherein the complementary spb member is pre-treated with a spc to remove non-specific binding elements associated with the complementary sbp member.

7. An assay procedure according to claim 6 wherein the pre-treatment spc is subsequently removed along with unbound labelled spc.

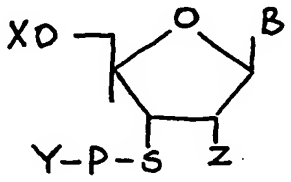
8. An assay procedure in which a labelled sbp member binds to a complementary sbp member and the binding is detected by a sps in conjunction with the label, characterised in that a first spc is used to remove non-specific binding elements from said complementary sbp member while a second spc is used for capture of the complementary sbp member.

9. A DNA or RNA analogue having a phosphodiester bridge including a cleavable phosphorus-sulphur bond.

10. A DNA or RNA analogue according to claim 9 wherein the phosphorus-sulphur bond is chemically cleavable.

11. A DNA or RNA analogue according to claim 10 wherein the phosphorus-sulphur bond is cleavable by mild oxidative hydrolysis.

12. A compound of the formula



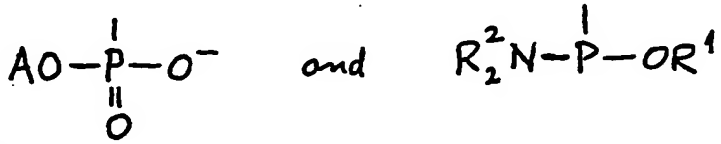
wherein: B is one of the common heterocyclic bases of DNA or RNA, or an analogue thereof;

X is a 5'-OH protecting group, preferably dimethoxytrityl;

Y is one or more atoms linked to the phosphorus atom, preferably including oxygen, hydrogen and nitrogen, and capable of forming an ester linkage with a nucleotide 5'-hydroxy group;

Z is hydrogen or OR, where R is hydrogen or a 2'-OH protecting group.

13. A compound of claim 12 wherein -P-Y is selected from:



wherein:

A is a protecting group, preferably an aryl

protecting group;

R^1 is an aliphatic protecting group, preferably cyanoethyl or methyl; and

R^2 is an alkyl group, preferably methyl, ethyl or propyl.

14. The use of a compound of claim 12 or claim 13 in the formation of a DNA or RNA analogue of any one of claims 9 to 11.

15. An assay procedure involving the capture of target nucleic acid by a nucleic acid probe immobilized on a solid phase component (spc), characterised in that the nucleic acid probe comprises a DNA or RNA analogue of any one of claims 9 to 14, and is subsequently released from the spc by cleavage of said phosphorus-sulphur bond.

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